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By: Lynnea B. Anderson

Our Docket No.: 50225-8034.US04

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:

Singh *et al.*

EXAMINER: Unknown

SERIAL No.: Not yet Assigned

ART UNIT: Unknown

FILED: Filed Herewith

FOR: **SAMPLE EVAPORATIVE CONTROL**

Preliminary Amendment

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified patent application, please amend the above-identified patent application as follows:

In the specification

On page 1, replace the first paragraph with the following paragraph:

-- This application is a continuation of Application Serial No. 09/965,448, filed September 27, 2001, now pending, which is a continuation of Application Serial No. 09/568,786, filed May 10, 2000, now pending, which is a continuation-in-part of Application Serial No. 09/470,677, filed December 23, 1999, now pending, and claims priority to provisional applications 60/133,448, filed on May 11, 1999 and 60/140,180, filed June 18, 1999, all of which disclosures are incorporated herein by reference. --

On page 14, replace the paragraph starting on line 14 with the following paragraph:

-- One may also have one or a multiplicity of vertical capillary channels comprising a terminal region having a larger cross-sectional area than the capillary channel which may comprise a non-wettable region at or above the interface between the terminal

region and the channel. The capillary would be placed in a reservoir to replenish liquid lost from the zone formed in the terminal region. As one added new liquid to the terminal region, initially the meniscus would be raised. Both evaporation and movement of the meniscus downward would occur, so that displacement of solution containing an active component would be minimized, keeping the volume of the zone minimal. The terminal region could be cylindrical, conical, or the like. Generally, the capillary channel would be circular, so that the terminal region would have at least about 1.2 times the diameter of the capillary channel, frequently at least about 1.5 times the diameter of the capillary channel and up to about 20 times.--

On page 30, replace the paragraph starting on line 14 with the following paragraph:

-- In many situations one may wish to separate constituents of an assay mixture. Where the substrate and product of an enzyme assay or chemical assay both provide the same signal, e.g. fluorescence, but have different mobilities, the substrate and product may be readily determined by using electrophoresis. Where multiplexed reactions are performed in the zone, one will have an interest in detecting the plurality of events that may have occurred. For example, one may have a plurality of reagents carrying electrophoretic tags (labels which have different mobilities in electrophoresis), where the result of the process in the zone is to release an electrophoretic tag in the presence of a target moiety. Where there may be a plurality of target moieties in the sample, the ability to detect the presence of the target moieties by the separation of released electrophoretic tags greatly enhances the simplicity with which the process may be carried out. Since the entire process may be automated, the addition of the assay components, the processing of the assay, the movement of the assay components into the electrokinesis system and the separation, confusion between samples is substantially eliminated, direct comparisons are achieved between samples and controls, component handling is minimized and more accurate results can be obtained.--

On page 34, replace the paragraph starting on line 7, with the following paragraph:

-- In Fig. 2B, liquid 220b is introduced into the wells 208b and 210b. In the present configuration, the liquid is indicated as being the same, but with different protocols the liquid could be different. The liquid 220b from the wells 208b and 210b moves by capillary action into channel 206b and halts at chamber 216b, due to the absence of capillarity at the chamber 216b. A sample may then be added to chamber 216b, which will wet the surface 218b. Where the sample is small enough, it will not contact the inlet ports 222b and 224b of channel 206b. Depending upon the nature of the solvent added to the chamber 216b and the time interval in which the solvent is allowed to stand, all or a portion of the solvent may evaporate, so that upon total evaporation, only a solvent free liquid or solid will be present.--

On page 40, replace the paragraph bridging pages 40 and 41 with the following:

-- The device has an upper plate 740 and a lower plate 742. The lower plate 742 has channels 716 and 720, which connect buffer reservoir 718 and waste reservoir 722 with zone enclosure 704, where the channel provides solution under the upper portion of the zone enclosure 712 with liquid from the channels 7716 and 720. While the diameters and the reservoirs are shown as approximately equal in Fig. 7B, this is for illustration. In practice, the zone enclosure diameter would normally not be greater, usually smaller than the reservoir diameters. In this case, by having a non-wettable wall 741 in the zone enclosure 708 , a convex meniscus 712 is observed and the height to which the liquid in the zone can rise is restricted.--

In the claims

Please cancel claims 1-38 without prejudice, and add new claims 39-51 as follows:

--39. A microfluidics device for performing an assay involving eletrophoretically separable assay components, comprising:

- (a) a solid substrate,
- (b) a microstructure unit having

- (i) a sample well having an interior end surface and an exposed opening, and a wall surface extending therebetween, said sample well being adapted to receive a volume of an assay solution,
- (ii) a first reservoir for holding a liquid,
- (iii) a first channel extending between the first reservoir and the sample well for carrying liquid in the first reservoir to the sample well,
- (iv) a side channel extending between the sample well and a second reservoir in the microstructure, and
- (v) an analysis channel that extends between third and fourth reservoirs in the microstructure and intersects the side channel, forming a sample loading region therewith,
- (c) electrodes adapted to be placed in said first, second, third, and fourth reservoirs, to contact liquid in the associated reservoirs, and
- (d) a control unit operatively connected to said electrodes for applying a selected voltage potential across the first and second reservoirs to move assay components in the sample well electrokinetically into the sample loading region via the side channel, and for applying a selected voltage potential across the third and fourth reservoirs to move assay components in the sample loading region electrokinetically along the analysis channel to separate the electrophoretically separable assay components,
- wherein, in operative condition, said first reservoir functions as a liquid reservoir for replenishing liquid in said sample well lost by evaporation during an assay, and as an electrolyte reservoir adapted to receive an electrode, when applying a voltage potential across the first and second reservoirs.

40. The device of claim 39, wherein said side channel has a wall surface with a net charge, such that applying a voltage potential across said first and second reservoirs, with an electrolyte contained in the side channel, is effective to move liquid in the first channel by electro-osmotic flow.

41. The device of claim 39, wherein the microstructure further includes a fifth reservoir connected to said sample well through a second channel, for holding a liquid used to replenish liquid lost from the sample well during an assay.

42. The device of claim 40, wherein:

the sample well has at least one cross-sectional area greater than that of said first channel and an interior border disposed within the sample well and spaced from the exposed opening, intermediate the opening and interior surface, wherein liquid placed in the sample well through the opening, or introduced therein through the first channel, forms a sample volume having a meniscus created by the border, below the exposed opening, and

the sample volume is maintained substantially constant, as liquid is added to the sample volume through the opening, by liquid flow through the first channel toward the first reservoir, and as solvent evaporates from the sample volume, by liquid flow from the first reservoir through the first channel toward the sample well.

43. The device of claim 42, wherein the sample well has different cross-sectional areas on progressing from the interior surface to the exposed opening.

44. The device of claim 42, wherein the border is a wettable/nonwettable border formed on the wall surface.

45. The device of claim 42, wherein the border is a sharp change in the direction of the wall surface.

46. A method of conducting a microvolume assay involving eletrophoretically separable assay components, comprising the steps of:

(a) placing sample components in a sample well,

(b) replenishing liquid lost by evaporation in the sample well by liquid flow from a first reservoir through a first channel into said assay well,

(c) moving sample components in the sample well electrokinetically along a side channel connecting the sample well to a second reservoir, by applying a voltage potential across the first and second reservoirs,

(d) by said moving, loading sample components in a sample-loading region formed at the intersection of said side channel and analysis channel,

(e) separating sample components contained in the sample-loading region by electrophoretic movement of the components along said analysis channel, and

(f) detecting the separated components in said analysis channel.

47. The method of claim 46, wherein said placing includes adding liquid sample to the sample well and, following said adding, drying the liquid to deposit dry reagents within the sample well.

48. The method of claim 46, wherein the sample well has an exposed opening and an interior border disposed within the well and spaced from the exposed opening, and wherein said placing includes forming in the sample well a sample volume having a meniscus created by the border and disposed below the opening.

49. The method of claim 48, wherein said placing further includes adding liquid sample reagent to the meniscus, through the opening, and allowing the meniscus to equilibrate by liquid flow from the sample volume through the first channel to the first reservoir.

50. The method of claim 49, wherein said replenishing includes maintaining the sample volume substantially constant as said evaporation occurs.

51. The method of claim 46, wherein said moving is by electro-osmotic flow.

REMARKS

Entry of the above newly submitted claims and the following remarks are respectfully requested.

Attached hereto is a marked-up version of the changes made to the specification and claims. The attached pages are captioned **"Version with markings to show changes made."**

I. Amendments

The specification has been amended to correct typographical errors and inconsistencies.

Claim 39 is directed to a *microfluidics device*, as described in the specification at the cites indicated. The device, which is described generally on page 5, lines 17-31 and page 6, line 28 to page 7, line 8, comprises:

(a) *a solid substrate*, as disclosed, for example, on page 32, lines 16-18 with respect to Fig. 1,

(b) *a microstructure unit*, as disclosed, for example, on page 5, lines 8-10, and page 32, line 16 to page 33, line 8 with respect to Fig. 1, *having*

(i) *a sample well having an interior end surface and an exposed opening*, as disclosed, for example, on page 5, lines 4-8 and on page 9, line 15 to page 10, line 7, *and a wall surface extending therebetween*, as disclosed, for example, on page 5, lines 17-29 and page 33, lines 10-12 with respect to Fig. 1,

said sample well being adapted to receive a volume of an assay solution, as disclosed, for example, on page 2, line 30 to page 3, line 4, page 15, lines 15-19, page 4, line 31 to page 5, line 14, and page 6, lines 1-7,

(ii) *a first reservoir for holding a liquid*, as disclosed, for example, on page 5, lines 8-12, and page 10, lines 8-11, *and*

(iii) *a first channel extending between the first reservoir and the sample well for carrying liquid in the first reservoir to the sample well*, as disclosed, for example, on page 5, lines 8-12, and page 10, lines 8-11,

(iv) *a side channel extending between the sample well and a second reservoir in the microstructure, as disclosed, for example, on page 29, lines 4-6 and 15-18, and*

(v) *an analysis channel that extends between third and fourth reservoirs in the microstructure and intersects the side channel, forming a sample loading region therewith, as disclosed, for example, on page 29, lines 7-9 and page 29, line 15 to page 30, line 13,*

(c) *electrodes adapted to be placed in said first, second, third, and fourth reservoirs, to contact liquid in the associated reservoirs, as disclosed, for example, on page 29, line 30 to page 30, line 9, and page 30, line 29 to page 31, line 3, and*

(d) *a control unit operatively connected to said electrodes for applying a selected voltage potential across the first and second reservoirs to move assay components in the sample well electrokinetically into the sample loading region via the side channel, as disclosed, for example, on page 31, lines 2-9; page 29, lines 4-9, and for applying a selected voltage potential across the third and fourth reservoirs to move assay components in the sample loading region electrokinetically along the analysis channel to separate the electrophoretically separable assay components, as disclosed, for example, on page 30, lines 5-13,*

wherein, in operative condition, said first reservoir functions as a liquid reservoir for replenishing liquid in said sample well lost by evaporation during an assay, as disclosed, for example, at page 5, lines 10-12 and lines 17-22, and as an electrolyte reservoir adapted to receive an electrode, when applying a voltage potential across the first and second reservoirs, as disclosed, for example, on page 46, lines 16-31 with respect to Fig. 12.

Claim 40 adds the further limitation to the device of claim 39 that *said side channel has a wall surface with a net charge, such that applying a voltage potential across said first and second reservoirs, with an electrolyte contained in the side channel, is effective to move liquid in the first channel by electro-osmotic flow, as disclosed, for example, on page 29, lines 6-7 and page 18, line 18-28.*

Claim 41 adds the further limitation to the device of claim 39 that *the microstructure further includes a fifth reservoir connected to said sample well through a second channel, for holding a liquid used to replenish liquid lost from the sample well during an assay, as disclosed, for example, on page 43, line 19 to page 44, line 12 with respect to Fig. 10.*

Claim 42 adds the further limitations to the device of claim 40 that:

the sample well has at least one cross-sectional area greater than that of said first channel, as disclosed, for example, on page 7, lines 23-27, and an interior border disposed within the sample well and spaced from the exposed opening, intermediate the opening and interior surface, as disclosed, for example, on page 5, lines 17-22, and page 42, lines 20-27 with respect to Fig. 8, wherein liquid placed in the sample well through the opening, or introduced therein through the first channel, as disclosed, for example, on page 10, lines 27-31; page 11, lines 25-27; page 12, lines 16-21; page 13, line 12 to page 14, line 6; and page 16, lines 17-28, forms a sample volume having a meniscus created by the border, below the exposed opening, as disclosed, for example, on page 6, lines 1-8 and page 7, lines 13-29, and

the sample volume is maintained substantially constant, as liquid is added to the sample volume through the opening, by liquid flow through the first channel toward the first reservoir, and as solvent evaporates from the sample volume, by liquid flow from the first reservoir through the first channel toward the sample well, as disclosed, for example, on page 5, lines 10-12 and lines 17-22, and page 11, lines 21-24..

Claim 43 adds the further limitation to the device of claim 42 that *said sample well has different cross-sectional areas on progressing from the interior surface to the exposed opening, as disclosed at, for example, Fig. 1 and Fig. 2.*

Claim 44 adds the further limitation to the device of claim 42 that *the border is a wettable/nonwettable border formed on the wall surface, as disclosed at, for example, page 5, lines 17-31; page 6, line 28 to page 7, line 8; and page 33, lines 9-13 with respect to Fig. 1.*

Claim 45 adds the further limitation to the device of claim 42 that *the border is a sharp change in the direction of the wall surface*, as disclosed at, for example, page 5, lines 17-19, and page 6, line 28 to page 7, line 8.

Claim 46 is directed to a *method of conducting a microvolume assay involving electrophoretically separable assay components*, as described in the specification at the cites indicated. The method, which is described generally at page 29, lines 1-14; page 30, line 14 to page 31, line 19; and page 22, line 30 to page 23, line 12, comprises:

- (a) *placing sample components in a sample well*, as disclosed, for example, on page 10, line 27 to page 12, line 21, and page 40, lines 9-13,
- (b) *replenishing liquid lost by evaporation in the sample well by liquid flow from a first reservoir through a first channel into said assay well*, as disclosed, for example, on page 5, lines 8-12 and page 10, lines 8-11,
- (c) *moving sample components in the sample well electrokinetically along a side channel connecting the sample well to a second reservoir, by applying a voltage potential across the first and second reservoirs*, as disclosed, for example, on page 29, line 15 to page 30, line 5,
- (d) *by said moving, loading sample components in a sample-loading region formed at the intersection of said side channel and analysis channel*, as disclosed, for example, on page 29, line 27 to page 30, line 5,
- (e) *separating sample components contained in the sample-loading region by electrophoretic movement of the components along said analysis channel*, as disclosed, for example, on page 30, lines 5-11, and
- (f) *detecting the separated components in said analysis channel*, as disclosed, for example, on page 30, lines 11-28.

Claim 47 adds the further limitation to the method of claim 46 that *said placing includes adding liquid sample to the sample well and, following said adding, drying the liquid to deposit dry reagents within the sample well*, as disclosed at, for example, page 34, lines 11-15 with respect to Fig. 2; page 16, lines 17-18; and page 19, lines 19-21.

Claim 48 adds the further limitation to the method of claim 46 that *the sample well has an exposed opening and an interior border disposed within the well and spaced from the exposed opening*, as disclosed at, for example, page 5, lines 4-8; page 9, line 15 to page 10, line 7; page 41, lines 3-5 with respect to Fig. 7B; and page 42, lines 20-27 with respect to Fig. 8B, and that *said placing includes forming in the sample well a sample volume having a meniscus created by the border and disposed below the opening*, as disclosed, for example, at page 6, lines 1-8, and page 7, lines 13-29.

Claim 49 adds the further limitation to the method of claim 48 that *said placing further includes adding liquid sample reagent to the meniscus, through the opening, and allowing the meniscus to equilibrate by liquid flow from the sample volume along the first channel toward the first reservoir*, as disclosed, for example, on page 5, lines 17-22; page 6, lines 7-10; and page 11, lines 21-24.

Claim 50 adds the further limitation to the method of claim 49 that *said replenishing includes maintaining the sample volume substantially constant as said evaporation occurs*, as disclosed, for example, on page 5, lines 10-14 and page 10, lines 8-9.

Claim 51 adds the further limitation to the method of claim 46 that *said moving is by electro-osmotic flow*, as disclosed, for example, on page 29, lines 6-7.

No new matter has been added by these amendments.

II. Consideration of prior art

Applicant has reviewed the documents cited in the accompanying IDS 1449 form. Although certain various microfluidics devices and methods for conducting microvolume assays have been disclosed in the prior art (see the accompanying IDS 1449 form), none

Version with Markings to Show Changes Made

This application is a continuation of Application Serial No. 09/965,448, filed September 27, 2001, now pending, which is a continuation of Application Serial No. 09/568,786, filed May 10, 2000, now pending, which is a continuation-in-part of Application Serial [no.]No. 09/470,677, filed December 23, 1999, now pending, and claims priority to provisional applications 60/133,448, filed on May 11, 1999 and 60/140,180, filed June 18, 1999, [which] all of which disclosures are incorporated herein by reference.

One may also have one or a multiplicity of vertical capillary channels comprising a terminal region having a larger cross-sectional area than the capillary channel which may comprise a non-wettable region at or above the interface between the terminal region and the channel. The capillary would be placed in a reservoir to replenish liquid lost from the zone formed in the terminal region. As one added new liquid to the terminal region, initially the meniscus would be raised. Both evaporation and movement of the meniscus downward would occur, so that displacement of solution containing an active component would be minimized, keeping the volume of the zone minimal. The terminal region could be cylindrical, conical, or the like. Generally, the capillary channel would be circular, so that the terminal region would have at least about 1.2 times the diameter of the capillary channel, frequently at least about 1.5 times the diameter of the capillary channel and up to about 20 times.[.]

In many situations one may wish to [separation] separate constituents of an assay mixture. Where the substrate and product of an enzyme assay or chemical assay both provide the same signal, e.g. fluorescence, but have different mobilities, the substrate and product may be readily determined by using electrophoresis. Where multiplexed reactions are performed in the zone, one will have an interest in detecting the plurality of events that may have occurred. For example, one may have a plurality of reagents carrying electrophoretic tags (labels which have different mobilities in electrophoresis), where the result of the process in the zone is to release an electrophoretic tag in the presence of a target moiety. Where there may be a plurality of target moieties in the sample, the ability to detect the presence of the target moieties by the separation of released electrophoretic tags greatly enhances the simplicity with

which the process may be carried out. Since the entire process may be automated, the addition of the assay components, the processing of the assay, the movement of the assay components into the electrokinesis system and the separation, confusion between samples is substantially eliminated, direct comparisons are achieved between samples and controls, component handling is minimized and more accurate results can be obtained.

In Fig. 2B, liquid 220b is introduced into the wells 208b and 210b. In the present configuration, the liquid is indicated as being the same, but with different protocols the liquid could be different. The liquid 220b from the wells 208b and 210b moves by capillary action into channel 206b and halts at chamber 216b, due to the absence of capillarity at the chamber [206b] 216b. A sample may then be added to chamber 216b, which will wet the surface 218b. Where the sample is small enough, it will not contact the inlet ports 222b and 224b of channel 206b. Depending upon the nature of the solvent added to the chamber 216b and the time interval in which the solvent is allowed to stand, all or a portion of the solvent may evaporate, so that upon total evaporation, only a solvent free liquid or solid will be present.

The device has an upper plate 740 and a lower plate 742. The lower plate 742 has channels 716 and 720, which connect buffer reservoir 718 and waste reservoir 722 with zone enclosure 704, where the channel provides solution under the upper portion of the zone enclosure 712 with liquid from the channels 7716 and 720. While the diameters and the reservoirs are shown as approximately equal in Fig. 7B, this is for illustration. In practice, the zone enclosure diameter would normally not be greater, usually smaller than the reservoir diameters. In this case, by having a non-wettable wall [746] 741 in the zone enclosure 708, a convex meniscus 712 is observed and the height to which the liquid in the zone can rise is restricted.